

MR 280349



October 26, 2004

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CONFIDENTIAL

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Document Processing Center (7407)  
Office of Pollution, Prevention and Toxics  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N. W.  
Washington, DC 20460  
Attention: Section 8(e) Coordinator

Re: **TSCA Section 8(e) Submissions**

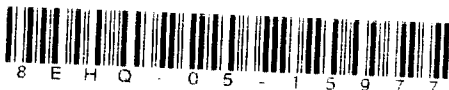
Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included a master index for these studies identifying the study title, test substance and CAS number. A Confidential Business Information (CBI) version of this index and the studies also is being submitted today pursuant to EPA procedures. 3M has not provided CBI substantiation with this submission, but would be willing to do so at the Agency's request.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Very truly yours,



*Katherine E. Reed* (9.7.)

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Staff Vice President  
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**Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004**  
**(Confidential Business Information Redacted)**

Title	Substance	CAS Information
Aquatic Toxicity Data Sheet: 48hr <i>Daphnia Magna</i>	1,4-dioxane, heptadecafluoro-1-octanesulfonic acid; linear n-ethyl perfluorooctanesulfonamide; n-ethylperfluorooctanesulfonamidoethyl alcohol; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([heptadecafluorooctyl)sulfonylamino]ethyl-, omega-hydroxy-; [ethyl]([nonafluorobutyl)sulfonylamino]ethyl-, omega-hydroxy-; [ethyl]([undecafluoropentyl)sulfonylamino]ethyl-, omega-hydroxy-; [ethyl]([tridecafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([pentafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy-; [ethyl]([tridecafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([undecafluoropentyl)sulfonylamino]ethyl-, omega-hydroxy-; polyethylene glycol; water	1,4-dioxane (123-91-1); heptadecafluoro-1-octanesulfonic acid (1763-23-1); linear n-ethyl perfluorooctanesulfonamide (4151-50-2); n-ethylperfluorooctanesulfonamidoethyl alcohol (1691-99-2); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([heptadecafluorooctyl)sulfonylamino]ethyl-, omega-hydroxy- (29117-08-6); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([nonafluorobutyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-79-3); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([pentafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-81-7); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([tridecafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy- (56372-23-7); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([undecafluoropentyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-80-6); polyethylene glycol (25322-68-3); water (7732-18-5)
Multigeneration Daphnid Life Cycle Test	1,4-dioxane, heptadecafluoro-1-octanesulfonic acid; linear n-ethyl perfluorooctanesulfonamide; n-ethylperfluorooctanesulfonamidoethyl alcohol; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([heptadecafluorooctyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([nonafluorobutyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([pentafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([tridecafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy-; poly(oxy-1,2-ethanediyl), alpha-12- [ethyl]([undecafluoropentyl)sulfonylamino]ethyl-, omega-hydroxy-; polyethylene glycol; water	1,4-dioxane (123-91-1); heptadecafluoro-1-octanesulfonic acid (1763-23-1); linear n-ethyl perfluorooctanesulfonamide (4151-50-2); n-ethylperfluorooctanesulfonamidoethyl alcohol (1691-99-2); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([heptadecafluorooctyl)sulfonylamino]ethyl-, omega-hydroxy- (29117-08-6); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([nonafluorobutyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-79-3); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([pentafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-81-7); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([tridecafluorohexyl)sulfonylamino]ethyl-, omega-hydroxy- (56372-23-7); poly(oxy-1,2-ethanediyl), alpha-12-[ethyl]([undecafluoropentyl)sulfonylamino]ethyl-, omega-hydroxy- (68298-80-6); polyethylene glycol (25322-68-3); water (7732-18-5)
Aquatic Invertebrate Testing - Alkyltins LR 8024-1  Aquatic Invertebrate Testing - Decosheen Material (LR-8052)  R Scratch Remover (Fathead Minnow)  S Scratch Remover (Fathead Minnow)  Octanol Water Partition Coefficient	Alkyltins: dibutyltin laurate and dibutyltin-di(2 ethylhexoate)  Decosheen Ribbon Materials and pigments: Decosheen Blue in Green Ceres Blue ZV; Decosheen Gold Paste Pigment; Decosheen Royal Blue, Solvent Blue  55-65% Water; 20-30% Stoddard Solvent; 1-5% Sodium Silicate; 1-5% Potassium Hydroxide; 0.1-3% Nonylphenoxypoly(oxyethylene)ethanol  60-70% Water; 20-30% Stoddard Solvent; 1-5% Sodium Silicate; 0.1-3% Turgitol NP-33 N-methylperfluorooctane sulfonamidoethanol	Dibutyltin laurate (CAS 77-58-7); Dibutyltin-di(2 ethylhexoate) (CAS 2781-10-4)  Decosheen Blue in Green, <del>Ceres Blue ZV</del> (CAS 61814-09-3); Decosheen Royal Blue, Solvent Blue <del>(CAS 61814-09-3)</del> ; Decosheen Gold Paste Pigment (CAS Number <del>Unkown</del> )  Water (CAS 7732-18-5); Stoddard Solvent (CAS 8052-41-3); Sodium Silicate (CAS 1344-09-8); Potassium Hydroxide (CAS 1310-58-3); Nonylphenoxypoly(oxyethylene)ethanol (CAS 9016-45-9)  Water (CAS 7732-18-5); Stoddard Solvent (CAS 8052-41-3); Sodium Silicate (CAS 1344-09-8); Turgitol NP-33 (CAS 9016-45-9) CAS 2448-09-7

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004  
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Title	Substance	CAS Information
CoCl <sub>2</sub> .6H <sub>2</sub> O as Co <sup>2+</sup> Toxicity to Microtox Reagent	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1
Activated Sludge Respiration Inhibition Test on CoCl <sub>2</sub> .6H <sub>2</sub> O as Co ion	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1
Acute Toxicity of CoCl <sub>2</sub> .6H <sub>2</sub> O as Co ion to <i>Daphnia magna</i> under Static Exposure Conditions	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1
Acute Toxicity of CoCl <sub>2</sub> .6H <sub>2</sub> O as Co ion to Fathead Minnow under Static Exposure Conditions	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1
Freshwater Algae Growth Inhibition Test	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1
<i>Daphnia magna</i> 21-Day Chronic Reproduction Study	N-ethylperfluorooctane sulfonamidoethanol	CAS 1691-99-2
Plant Growth Effects of [ ]	[ ]	[ ]
Final Report ( <i>Daphnia</i> and Microtox)	Monomethyl ether of hydroquinone	CAS 150-76-5
Microtox Test Results	2 Ethylhexyl Acrylate; Isooctyl Acrylate Monomer; 2-Methylbutyl acrylate; Methyl Isoamyl acrylate; Isooctyl Acrylate	2 Ethylhexyl Acrylate (CAS 103-11-7); Isooctyl Acrylate Monomer (CAS 29590-42-9) 2-Methylbutyl acrylate (CAS 44914-03-6); Methyl isoamyl acrylate (CAS 18993-92-1); Isooctyl Acrylate (CAS 29590-42-9)
Phytotoxicity Test Results	[ ]	[ ]

**Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004**  
**(Confidential Business Information Redacted)**

Title	Substance	CAS Information
Plant Toxicity Comparison, Young Seeding Growth	[ ]	[ ]
<i>Ceriodaphnia dubia</i> Survival and Reproduction exposed to Opequon Creek Water Spiked with BETZ 1110 Polymer (November 4, 1987 sample) for seven days under static renewal conditions	BETZ 1110: Non-3M Product - Chemical composition not provided to 3M by manufacturer	MSDS provided by manufacturer states product is "not hazardous" and not "considered to be a carcinogen"
<i>Ceriodaphnia dubia</i> Survival and Reproduction exposed to Opequon Creek Water Spiked with Betz 1138 Polymer (November 4, 1987 sample) for seven days under static renewal conditions	BETZ 1138: Non-3M Product - Chemical composition not provided to 3M by manufacturer	MSDS provided by manufacturer states product is "not hazardous" and not "considered to be a carcinogen"
Toxicity of 1,6 - Hexanediol Diacrylate to <i>Daphnia magna</i>	1,6 Hexanediol diacrylate	CAS 13048-33-4
<i>Daphnia magna</i> Chronic Bioassay Under Static Renewal Conditions	Methyl isoamyl acrylate	CAS 18993-92-1
Estimating the Chronic Toxicity of Nalclear 7177 to <i>Ceriodaphnia</i> Survival and Reproduction Using Short-Term Tests	Nalclear 7177 wastewater treatment acrylamide/acrylate polymer - Chemical composition not provided to 3M by manufacturer	CAS Information not provided to 3M by manufacturer
Acute Toxicity of Isooctyl Acrylate to <i>Daphnia magna</i>	Isooctyl Acrylate Monomer	CAS 29590-42-9
Static Acute Toxicity of [ ] to the <i>Daphnid, Daphnia magna</i>	Tolyltriazole	CAS 29385-43-1
Static Acute Toxicity of [ ] to the <i>Alga, Selenastrum capricornutum</i>	Tolyltriazole	CAS 29385-43-1
Static Acute Toxicity of [ ] to the <i>Daphnid, Daphnia magna</i>	[ ]	[ ]
Static Acute Toxicity of [ ] to the Fathead Minnow, <i>Pimephales promelas</i>	[ ]	[ ]
Static Acute Toxicity of [ ] to the <i>Daphnid, Daphnia magna</i>	water; propylene-tetrafluoroethylene polymer; tert-butyl alcohol	water (7732-18-5); propylene-tetrafluoroethylene polymer (27029-05-6); tert-butyl alcohol (75-65-0)

**Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004**  
**(Confidential Business Information Redacted)**

Title	Substance Information	CAS Information
Isooctyl acrylate: Fish, Acute Toxicity Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: <i>Daphnia</i> sp. Acute Immobilization Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: Alga, Growth Inhibition Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Isooctyl Acrylate: <i>Daphnia</i> sp. Reproduction Test	Isooctyl Acrylate Monomer	CAS 29590-42-9
Acute Toxicity of [ ] to the mysid, <i>Mysidopsis bahia</i>	[ ]	[ ]
Final Report (Microtox)	[ ]	[ ]
Determination of the Partition Coefficient (N-Octanol/Water) of T-5896 by High Performance Liquid Chromatography (HPLC)	N-methyl perfluorooctane sulfonamido ethanol; N-methyl perfluorooctane sulfonamidoethyl acrylate	N-methyl perfluorooctane sulfonamido ethanol (CAS 25268-77-3); N-methyl perfluorooctane sulfonamidoethyl acrylate (CAS 24448-09-7)
OECD Activated Sludge Respiration Inhibition Test Results	N-Dodecyltrimethylammonium chloride	CAS = 112-00-5
Final Report (Fish Acute Toxicity)	Mirataine CB (30% Cocamidopropyl betaine = Amides, coco, N-(3-dimethylamino)propyl), alkylation products with chloroacetic acid, Coco/Oleamidopropyl Betaine = 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., inner salt)	Cocamidopropyl betaine (CAS 70851-07-9); Coco/Oleamidopropyl Betaine (CAS 61789-40-0)
A Flow-Through Life-Cycle Toxicity Test With the Saltwater Mysid ( <i>Mysidopsis bahia</i> )	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Alga, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
An Early Life-Stage Toxicity Test With the Fathead Minnow ( <i>Pimephales promelas</i> )	Perfluorooctane sulfonate	CAS 1763-23-1
Lithium: Fish, Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Lithium: <i>Daphnia</i> , Acute toxicity Tests	Lithium Chloride	CAS 7447-41-8
Summary of Toxicity Testing on OSCI and OSF	Octane sulfonyl chloride and Octane sulfonyl fluoride	Octane sulfonyl fluoride (CAS 7795-95-1); Octane sulfonyl chloride (CAS 4063-63-5)
Toxicity to Microtox Test	Lauryldimethylamineoxide	CAS 1643-20-5

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on October 26, 2004  
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Title	Substance Identification	CAS Information
Ecotoxicological Testing of CoCl <sub>2</sub> .6H <sub>2</sub> O as Co <sup>2+</sup> ion (Seed Germination and Root Elongation)	Cobalt (as Co <sup>2+</sup> ion) (CoCl <sub>2</sub> .6H <sub>2</sub> O)	CAS 7791-13-1

ASCI Corporation/ASCI-Duluth  
Environmental Testing Division  
ASCI Report ID# 003-DMC-23M  
ASCI Study ID# 5030-003-08

**STUDY TITLE**

**ISOOCTYL ACRYLATE: *DAPHNIA* sp. REPRODUCTION TEST**

**DATA STANDARD**

**OECD GUIDELINE 202**

**AUTHORS**

**Joe Amato and Donald Mount**

**STUDY COMPLETED**

**March 31, 1993**

**TESTING FACILITY**

**ASCI Corporation  
ASCI-Duluth Environmental Testing Division  
112 East Second Street  
Duluth, MN 55805**

**TEL. NO. (218) 722-4040**

**PROJECT IDENTIFICATION NUMBERS**

**ASCI Study ID# 5030-003-08**

**3M Company Study ID# J2774**

# CERTIFICATION OF GOOD LABORATORY PRACTICE COMPLIANCE

To the best of my knowledge, this study was conducted in accordance with OECD Good Laboratory Practice Standards (OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice 1981).

Study Director: [Signature] Date: 3/31/93  
Joe Amato  
ASCI Corporation/ASCI-Duluth  
Environmental Testing Division

Based on the signatures of the Study Director and the Quality Assurance Auditor, this study, to the best of our knowledge, was conducted in accordance with OECD Good Laboratory Practice Standards (OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice 1981).

Sponsor: [Signature] Date: 4-5-93  
Submitter: [Signature] Date: 4/5/93

# STATEMENT OF QUALITY ASSURANCE

The study data were reviewed by the ASCI Quality Assurance Unit to assure that standard operating procedures and guidelines used to conduct this study were followed, and this report is an accurate reflection of the raw data. The types of audits performed are listed in the following table.

Type of Audit for ASCI Study ID# 5030-003-08	Audit Date	Date Reported to Study Director and Management
Study Plan	12-17-1991	12-17-1991
In-Life Phase Biology	08-04-1992	08-04-1992
In-Life Phase Analytical Chemistry	08-06-1992	08-06-1992
In-Life Phase Biology & Analytical Chemistry	08-18-1992	08-18-1992
Raw Data and Draft Report	12-11-1992	12-11-1992
Final Report	03-31-1993	03-31-1993

  
Dinesh Vaishnav

Designated Quality Assurance Unit Staff

Date: 3/31/93

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## TERMINOLOGY

The following key terms to describe the test substance measured concentrations and the test substance effect on the test organisms are frequently used in this report:

### Test Substance Concentrations

Initial Measured Concentrations. Concentrations in fresh test solutions determined before dispensing in exposure chambers on days 2, 8, 15, and 20.

Initial Mean Measured Concentrations. The means of the initial measured concentrations during the entirety of the test.

Final Concentrations. Concentrations in spent solutions from individual replicates determined on days 3, 9, 16, and 21.

Mean Measured Concentrations. The pooled means of the initial measured concentrations from days 2, 8, 15, and 20, and the mean final concentrations.

### Test Substance Effect

EC50. The test substance estimated concentration, which in a specified time period should immobilize 50% of the test organisms when compared to the control value.

IC50. The test substance estimated concentration, which in a specified time period should cause a 50% reduction in fecundity of the test organisms when compared to the control value.

No Observed Effect Concentration (NOEC). The highest test substance concentration tested at which no significant reduction in survival or reproduction of the test organisms is observed when compared to the control value.

Lowest Observed Effect Concentration (LOEC). The lowest test substance concentration at which a significant reduction in survival or reproduction of the test organisms is observed when compared to the control value.

**STUDY SUMMARY TABLE**

<b>Study Title</b>	Isocetyl Acrylate: <i>Daphnia</i> sp. Reproduction Test
<b>Data Standard</b>	OECD Guideline 202 (OECD 1984), and Good Laboratory Practice standards as promulgated under the OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice (OECD 1981).
<b>Sponsor</b>	Rich Purdy, 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106; Tel No. (612) 778-5379.
<b>Sponsor's Representative</b>	Susan A. Beach, 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106; Tel No. (612) 778-7452.
<b>Testing Facility</b>	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 112 East Second Street, Duluth, MN 55805; Tel. No. (218) 722-4040.
<b>Study Director</b>	Joe Amato
<b>Designated QAU Staff</b>	Dinesh Vaishnav
<b>Testing Facility Director</b>	Donald Mount
<b>Study Initiation Date</b>	March 10, 1992.
<b>Test Dates</b>	Acute Definitive: January 21-23, 1992 Reproduction Definitive: July 28-August 18, 1992.
<b>Test Substance</b>	Isocetyl acrylate (CAS No. 29590-42-9, <del>Lot 3290</del> , Lot 3290), 99.7% acrylate (as determined by Sponsor NSF <del>Lot 3290</del> ), liquid.
<b>Test Organism</b>	<i>Daphnia magna</i> ; less than 24-h old neonates.

Test Description	<p>(1) Control, test, and monitoring chambers (each 400 ml volume) were prepared using 500-ml wide-mouth glass jars,</p> <p>(2) chambers were sealed with clear plastic sheets and each day the headspace in each chamber was flushed with oxygen to maintain DO,</p> <p>(3) chambers were incubated for 21 days, and exposure water chemistry parameters and test substance concentrations determined at appropriate time intervals,</p> <p>(4) stock solutions were prepared and analyzed daily,</p> <p>(5) test solutions were renewed daily,</p> <p>(6) test organisms were observed for immobilization and reproduction (effect), and</p> <p>(7) effect data were used to calculate EC50, IC50, and NOEC values, based on initial and mean measured test substance concentrations.</p>
Test Results	<p>(1) No EC50 values could be calculated for 1-day, 2-day, 4-day, or 7-day intervals because of insufficient effect,</p> <p>(2) 14-day EC50 was 2.93 mg/L based on initial measured concentrations and 1.99 mg/L based on mean measured concentrations,</p> <p>(3) 21-day EC50 was 2.62 mg/L based on initial mean measured concentrations and 1.61 mg/L based on mean measured concentrations,</p> <p>(4) 14-day IC50 based on initial measured concentration was 1.50 mg/L, and 0.97 mg/L based on mean measured concentrations,</p>

	<p>(5) 21-day IC50 value based on initial mean measured concentration was 1.72 mg/L and 1.02 mg/L based on mean measured concentrations,</p> <p>(6) 14-day NOEC values were 0.79 mg/L and 0.51 mg/L based on initial measured concentrations and mean measured concentrations, respectively, and</p> <p>(7) 21-day NOEC values were &lt; 0.20 mg/L based on initial mean measured concentrations and &lt; 0.13 mg/L based on mean measured concentrations.</p>
Location of Raw Data and Final Report	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 112 East Second Street, Duluth, MN 55805; Tel. No. (218) 722-4040.

## 1.0 INTRODUCTION

The test substance, isooctyl acrylate, is an ester primarily made from isooctanol and acrylic acid. It has negligible solubility in freshwater and its sub-lethal toxicity to *Daphnia* sp. is not known. The purpose of the present study was to determine the 14-day and 21-day NOEC, LOEC, and IC50 of the test substance for *Daphnia magna*. Also, the test substance 1-day, 2-day, 4-day, 7-day, 14-day, and 21-day EC50 values were determined based on immobilization of *D. magna*.

This study was conducted in accordance with OECD Guideline 202 (OECD 1984) and ASCI Study Plan No. OECD 202.C.

## 2.0 TEST METHODS

**2.1 Test Substance.** The test substance, isooctyl acrylate (CAS No. 29590-42-9, [ ] Lot 3290), was received at ASCI on October 3, 1991 in one amber glass bottle placed in a sealed metal container. The test substance was stored at room temperature as received.

The Sponsor had provided the test substance material safety data sheet and a written communication to ASCI. Accordingly, the test substance has negligible water solubility and 1 mm Hg vapor pressure at 50°C. Furthermore, the test substance is 99.75% acrylate, stable, and its biodegradation ranged from 59%-85% in five days. The Sponsor had also provided a method for analyzing the test substance. ASCI modified the test substance extraction procedure and validated the method. The Sponsor also has information that, based on the chemical structure, there will be essentially no dissociation of the test substance at environmental pH levels. The Sponsor suspects the test substance may have glass surface activity.

2.2 Test Substance Solutions. The test substance stock solution was prepared each day as follows:

- (1) Added 110  $\mu$ l of test substance to 8 L of well water contained in a 19 L preconditioned glass carboy;
- (2) Vigorously stirred the mixture at ambient temperature for 20 minutes with a mechanical stirrer;
- (3) Stopped stirring and held the mixture for an additional 20 minutes;

- (4) Siphoned and discarded the first 100 ml of the aqueous solution;
- (5) The remaining solution was considered the stock solution having a nominal concentration of 10 mg/L; and
- (6) The stock solution was analyzed each day for the test substance concentration. The analysis was performed using duplicate samples.

For this test, five test substance nominal concentrations were prepared in a geometric series from the test substance stock solution. Test substance nominal concentrations of 4 mg/L, 2 mg/L, 1 mg/L, 0.5 mg/L, and 0.25 mg/L were selected based on the 48-h EC50 value obtained from the *D. magna* definitive acute toxicity test. The appropriate dilutions to obtain the test substance daily nominal exposure concentrations were determined based on the mean value of the daily duplicate analyses of the test substance stock solution. The volume of the stock solution was adjusted each day to achieve the nominal concentrations. However, due to errors in calculating the test substance stock solution concentration, the test substance nominal concentrations on days 5 and 16 of the test deviated from the test substance nominal exposure concentrations listed above. On day 5 the dilutions prepared yielded nominal test substance concentrations of 3.7 mg/L, 1.8 mg/L, 0.9 mg/L, 0.5 mg/L,

and 0.23 mg/L. On day 16 the nominal test substance concentrations were 3.5 mg/L, 1.7 mg/L, 0.9 mg/L, 0.4 mg/L, and 0.22 mg/L. The pH of the test substance solutions was not adjusted.

**2.3 Test Organism.** Test organisms were *D. magna* neonates, less than 24-h old, obtained from gravid daphnids. The latter were obtained from a stock culture maintained at ASCI and were isolated 24 h before test initiation in dilution water containing food. Neither the stock culture organisms from which the gravid daphnids were obtained nor the test organisms (neonates) appeared diseased or stressed.

**2.4 Dilution Water.** Dilution water was shallow well water collected from the Two Harbors, (Minnesota) area. During the test, the water had a hardness of 128-170 mg/L (as CaCO<sub>3</sub>) and a pH of 7.9-8.6. The water was aerated for 24 h prior to using in the test.

The well water is analyzed annually and the most recent chemical analysis is provided in Appendix A.

**2.5 Exposure Chambers.** Exposure chambers were 500-ml, borosilicate-glass jars, each containing 400 ml of dilution water or test solution. During the test, the exposure chambers were kept

sealed, except when experimental observations and solution renewals were made. The chambers were sealed with plastic wrapping, and following solution renewal, the headspace in each chamber was flushed with sufficient oxygen to distend the plastic wrapping. The oxygen source was medical-grade, compressed, bottled gas having a purity of >99.5%. A pair of exposure chambers was assigned for each test replicate to facilitate solution renewals. For example, with the pair of test chambers assigned to control exposure, replicate A was used exclusively for that replicate for the duration of the test. The chamber sets were rotated on a daily basis, and the set containing final test solutions was cleaned immediately following solution renewal.

**2.6 Test Performance.** The test was conducted with four replicates each of a dilution water control and five test substance concentrations (total 24 exposure chambers). In addition, one monitoring chamber for each low, middle and high test substance concentration and dilution water control was setup for the sole purpose of measuring pH, dissolved oxygen concentration, specific conductivity, and temperature (total additional four monitoring chambers).

To begin the test, ten test organisms per exposure/monitoring

chamber were impartially distributed. The organisms were handled with a smooth bore pipet with an inside diameter of 5 mm. The test organisms were fed at each daily test solution renewal 3 ml of a mixture of yeast, Cerophyl<sup>®</sup>, and fermented trout chow (1,800 mg/L total solids), and 6 ml of an algal (*Selenastrum capricornutum*) suspension of  $3.5 \times 10^7$  cells/ml. The trout chow is analyzed annually and the most recent analysis is contained in Appendix A.

During the test, solutions were renewed daily, water temperature was maintained at  $21 \pm 1^\circ\text{C}$ , and daily photoperiod was maintained, using cool white fluorescent lamps, for 16-h light and 8-h dark periods. To avoid any stress to the animals, direct light to the test chambers was decreased by suspending an opaque sheet approximately 25 cm above the top of the test chambers. The light intensity determined at a later date under conditions identical to the test conditions was less than 10 ft-c.

Parental test organisms (F1) were observed daily for the test substance effect. For this, exposure chambers were first gently agitated and then observed to count the number of test organisms that did not swim within 15 seconds, that is, the number of test organisms affected by the test substance.

At each solution renewal, exposure chambers were observed to count both live young produced and any dead neonates. Subsequently, (1) the adult test organisms (F1 generation) were transferred to fresh test solutions, (2) the neonates (brood) were poured away, and (3) the presence of any eggs from which no young emerged was checked. Each time when observations were made, all immobilized parental test organisms were removed from the exposure chambers.

2.7 Determination of Water Chemistry Parameters. During the test, (1) water chemistry parameters of total hardness and alkalinity were determined at test initiation and at each solution renewal for the high test substance concentration and control, (2) specific conductivity was determined at test initiation and before each solution renewal, and (3) temperature, dissolved oxygen concentration, and pH were recorded at test initiation, and before and after each solution renewal. All measurements, except total hardness and alkalinity, were made using monitoring chambers. The total hardness and alkalinity were determined using samples of test solution/exposure water before the solutions were transferred to the exposure chambers.

2.8 Test Substance Analysis. The test substance concentrations in individual and composite samples were analyzed according to the

following schedule:

Type of Sample	Frequency of Sampling	Total Number of Samples Analyzed
Stock solution	Daily	21 samples (1 sample/day)
Test and control (initial solutions)	Days 2, 8, 15, and 20	24 parent solution samples (6 samples/day)
Test and control (final solutions)	Days 3, 9, 16, and 21	96 individual samples (24 samples/day)

The parent samples were collected from 4-L Erlenmeyer flasks which were used to hold the test solutions before they were delivered to the test chambers. Final solutions were collected directly from the exposure chambers immediately following biological observations and water chemistry determinations.

Stock solution analyses were performed daily. Each time, a well water blank, a well water spike, and duplicate stock solution samples were analyzed. For the spike solutions, the target concentration was 8.8 mg/L. This target spike concentration was obtained by spiking 1.0 ml of an acetone/test substance stock solution of 880 mg/L into 100 ml of well water contained in a 100 ml volumetric flask. Stock solution samples were collected directly into 100-ml volumetric flasks from the 19-L glass carboy

using a pre-conditioned glass siphon.

For analyzing the test substance concentrations in test solutions, 100 g (100 ml) samples were poured from the 4-L Erlenmeyer flasks or exposure chambers and placed in 125-ml brown glass bottles. The sample volumes were weighed using a top-loading balance. The samples were extracted and concentrated to 1 ml in methylene chloride. Sample concentration was performed under nitrogen stream before analyzing the test substance. Appendix B (Isocetyl acrylate: Method validation for analysis from water) contains details of the methods used for the test substance quantification.

**2.9 Treatment of Results.** The cumulative percentage of affected (immobilized) test organisms at each test substance concentration and exposure period was calculated in comparison to the control value. These data were then plotted against both the mean measured initial and mean measured test substance concentrations. The data were used to calculate the test substance 1-day, 2-day, 4-day, 7-day, 14-day, and 21-day EC50 values using trimmed Spearman-Kärber method (Hamilton et. al. 1977).

Reproduction data for 14 and 21 days were analyzed using a point estimation technique (Marcus and Holtzman 1988) to determine the

test substance IC50, that is, 50% inhibition of the mean number of young produced per female compared to control organism reproduction. Also when calculable, 95% confidence intervals were provided. The data analysis were performed using a computer software (Version 2.01, developed by ASCI Corporation) for estimating chronic toxicity by inhibition concentration percentage (ICp) techniques (Norberg-King 1988).

The 14-day and 21-day NOEC and LOEC values based on the *t*-statistics ( $p \leq 0.05$ ), with respect to both test organisms survival and reproduction, were determined using the TOXSTAT, Version 3.1 (University of Wyoming, Laramie, Wyoming 1989) software program.

### 3.0 RESULTS

Test organism immobilization data are in Table 1. Up to 7 days, no EC50 value could be calculated due to lack of test substance effect on organism survival. At duration intervals of 14 days and 21 days EC50 values were calculable, as 68% and 90% immobilization occurred in the 4 mg/L test substance nominal concentration. At 21 days, immobilization in the control and 0.25 mg/L, 0.5 mg/L, 1 mg/L, and 2 mg/L test substance nominal concentrations ranged from 5% to 10%.

Table 2 gives the data on live and dead young produced through 14 days and 21 days of the test. At 14 days, total live young produced ranged from 1 in the 4 mg/L test substance nominal concentration to 2,611 in the controls, and at 21 days live young produced ranged from 1 in the 4 mg/L test substance nominal concentration to 6,341 in the controls.

The 14-day test substance EC50 for the parental generation was 2.93 mg/L based on the initial measured concentrations from day 2 analyses. Initial measured concentrations for day 8 and final measured concentrations for day 9 were not included in the data analyses. The values obtained for day 8 were low and appeared to be incorrect as the final test substance measurements from these solutions (day 9) were comparable to the final values obtained on days 3, 16, and 21. Although the day 9 values appear to be correct, they were also excluded from calculating the mean concentrations so that the mean concentrations are not skewed toward the final concentration values.

Based on the initial mean measured concentrations calculated from days 2, 15, and 20 analyses, the 21-day EC50 was 2.62 mg/L. EC50's calculated from the mean measured concentrations at 14 days and 21 days were 1.99 mg/L and 1.61 mg/L, respectively. Due to

insufficient effect at days 1, 2, 4, and 7, no EC50's could be determined for these intervals (Table 3).

Inhibition concentrations (IC50's) based on the combined effects of reduced reproduction and survival are also presented in Table 3. The 14-day IC50 value was 1.50 mg/L based on the initial measured concentrations from day 2. The 21-day IC50 was 1.72 mg/L based on the mean initial test substance concentrations determined on days 2, 15, and 20.

Using the mean measured test substance concentrations calculated from days 2 and 3 analytical results, a 14-day IC50 value of 0.97 mg/L was obtained. The 21-day IC50, based on the mean measured test substance concentrations calculated from analyses on days 2, 3, 15, 16, 20, and 21, was 1.02 mg/L (Table 3).

NOEC's and LOEC's were determined for both reproduction and survival. Fourteen-day NOEC and LOEC values for reproduction were 0.79 mg/L and 0.19 mg/L based on the initial measured concentrations from day 2 analyses, and 0.51 mg/L and 0.11 mg/L based on means of day 2 initial and day 3 mean final analyses.

A definite value is not given for 21-day NOEC based on

reproduction, as live young produced for each test substance concentration was significantly less than the control value (Table 3).

The 14-day survival data were found to be non-homogeneous. Consequently, the NOEC and LOEC values were determined using the Steels Many-One Rank Test. Survival NOEC and LOEC values based on the day 2 initial test substance measured concentrations were 1.68 mg/L and 3.61 mg/L, respectively. A NOEC of 1.09 mg/L and a LOEC of 2.50 mg/L were determined based on analytical results from day 2 initial test substance concentrations and day 3 final test substance concentrations (Table 3).

NOEC and LOEC values for 21-day survival results were 1.79 mg/L and 3.79 mg/L based on the test substance mean initial measured concentrations obtained from analyses on days 2, 15, and 20.

The 21-day NOEC and LOEC values based on survival were 1.06 mg/L and 2.40 mg/L. These values were obtained using test substance mean measured concentrations calculated from analyses for initial concentrations from days 2, 15, and 20, and final concentrations from days 3, 16, and 21.

Daily mean stock solution concentrations and corresponding spike recoveries are presented in Table 4. Spiked solution recoveries ranged from 57.9% to 127.3%. Mean stock solution concentrations corrected for recoveries ranged from 9.1 mg/L to 13.7 mg/L.

The data for standard (deionized water), initial well water (not containing daphnid food), and final well water (containing daphnid food) spike recoveries are in Table 5. Well water not containing daphnid food was used as the spiking matrix for the initial test substance analyses because the initial test solutions were extracted prior to the addition of food. Well water containing daphnid food was used as the spiking matrix for the final test substance analyses to account for any effects the food might have on the test substance recovery from final test solutions.

The mean spike recovery from deionized water was  $87 \pm 10.1\%$ , from well water not containing daphnid food was  $86 \pm 7.1\%$ , and from well water containing daphnid food  $81 \pm 18.5\%$ . The values from days 8 and 9 were not included in the mean recovery calculations because the test substance measured concentrations from those days were not used in the determination of test substance exposure concentrations.

Test substance initial measured concentrations are in Table 6, and final measured concentrations are in Table 7. The test substance initial measured concentrations were corrected for the well water (not containing daphnid food) spike recovery for that day, and the final measured concentrations were corrected for well water (containing daphnid food) spike recovery obtained on that particular day.

The mean measured initial and mean measured test substance exposure concentrations used to determine 14-day and 21-day test endpoints are shown in Table 8.

Water chemistry values are in Tables 9-14. Table 9 shows the hardness of the control and 4 mg/L test substance nominal concentration ranged from 128 mg/L to 170 mg/L, and alkalinity (Table 10) ranged from 105 mg/L to 151 mg/L (both as  $\text{CaCO}_3$ ). The test temperatures (Table 11) were between 20.2°C and 22.4°C, and dissolved oxygen concentrations (Table 12) ranged from 4.5 mg/L to 9.0 mg/L. Table 13 shows pH values ranged from 7.6 to 8.6. Values given in Table 14 show test solution conductivities ranged from 280  $\mu\text{mhos/cm}$  to 381  $\mu\text{mhos/cm}$ .

In twelve (12) instances dissolved oxygen concentrations of the control, low and middle test substance concentrations were less than 60% (51%-59%) saturation at the test temperature (Table 12). All measured dissolved oxygen concentrations for the high test substance exposure were greater than 60% saturation. The low dissolved oxygen concentrations may be related to the number of adult organisms and neonates present in the test chamber rather than test substance concentration.

Table 15 is a description of the test acceptance QA criteria and results. Control organism survival, reproduction, and time for appearance of first brood met the acceptance QA criteria. The test duration was 21 days as specified. Twelve dissolved oxygen concentrations were below the set criteria, and pH deviations were observed on two occasions.

#### 4.0 DISCUSSION

The NOEC's and LOEC's from hypothesis testing are shown in Table 3. Based on reproduction, at 14 days the nominal 1 mg/L concentration was not different from the control, but the nominal 4, 2, 0.5, and 0.25 mg/L were significantly lower than the control. At 21 days,

all treatments were significantly lower than the control although the 1 mg/L treatment group was only slightly different.

Table 2 shows live young production, and for both 14 and 21 day sets of data, total live young production increased as the concentration increased for the 0.25, 0.5 and 1 mg/L treatments. This shows no dose response or a negative dose response, if one assumes the differences are real. In either case, any differences found by the hypothesis test cannot be attributed to treatment effects since the mandatory assumption for a toxicity test of a dose response is not met in this range of exposure. If the 0.25, 0.5, and 1 mg/L treatments are viewed as no effect, then the dose, response requirement is met for control, 2 mg/L and 4 mg/L treatments. Viewed in that way, the 2 mg/L test substance nominal concentration is the LOEC and the 1 mg/L is the NOEC. This NOEC and LOEC also fits the obvious break in the dose response curve between the 1 mg/L and 2 mg/L treatments.

The above argument is further strengthened by comparing young production in the various treatments to the mandatory 60 young per female in the controls for test acceptability. Young production at 2 mg/L was only 1.5 times the minimum young production; whereas, it was 2.4 times at 1 mg/L compared to 2.7 in the control. Again, a

sharp break between 1 mg/L and 2 mg/L test substance nominal concentrations occurred.

Statistical differences are totally dependant on the test variance and must be used with professional judgement. Primarily, because a dose response is not obtained from control to 1 mg/L exposure, the statistical differences cannot be attributed to treatment effects. If professional judgement is used, clearly the NOEC is 1 mg/L and the LOEC is 2 mg/L. Another strictly objective approach would be to use regression analyses which is a more appropriate way to analyze toxicity data of this type. The results of this approach are also shown in Table 3 as the IC50. This shows the 50% inhibition concentration between 1 mg/L and 2 mg/L, consistent with the professional judgement discussed above.

#### 5.0 CONCLUSIONS

An EC50 value could not be calculated from the test immobilization data until 14 days. The 14-day EC50 based on initial measured concentrations from day 2 was 2.93 mg/L, and based on mean measured concentrations was 1.99 mg/L. The 21-day EC50's based on initial mean measured concentrations and mean measured concentrations were 2.62 mg/L and 1.61 mg/L, respectively.

Fourteen and 21-day IC50's based on initial mean measured concentrations were 1.50 mg/L and 1.72 mg/L. Fourteen and 21-day IC50's based on mean measured concentrations were 0.97 mg/L and 1.02 mg/L, respectively.

#### 6.0 DEVIATIONS FROM APPROVED ASCI STUDY PLAN

- (1) Test substance 100% saturated stock solution was prepared in a 19-L glass carbon rather than a 6-L reaction flask in order to provide sufficient stock solution volume.
- (2) Stock solution was vigorously stirred for 20 minutes rather than gentle stirring.
- (3) Due to errors in calculating the test substance stock solution concentration, the nominal test substance exposure concentrations on days 5 and 16 of the test deviated from the test substance nominal exposure concentrations of 4 mg/L, 2 mg/L, 1 mg/L, 0.5 mg/L, and 0.25 mg/L. On day 5 the dilutions prepared yielded a nominal test substance concentration series of 3.7 mg/L, 1.8 mg/L, 0.9 mg/L, 0.5 mg/L, and 0.23 mg/L. On day 16 the nominal test substance concentration series was 3.5 mg/L, 1.7 mg/L, 0.9 mg/L, 0.4 mg/L, and 0.22 mg/L.
- (4) Dilution water hardness ranged from 128-170 mg/L CaCO<sub>3</sub>, instead of 170 mg/L-200 mg/L.

- (5) Dilution water pH ranged from 7.6-8.6 standard units rather than 7.5-8.5.
- (6) On day 11 of the test, final temperatures were 22.4°C, 0.4°C above the specified range.
- (7) Thirteen of 84 individual final dissolved oxygen measurements revealed concentrations below 60% of air saturation value at the test temperature. The concentrations detected below 60% saturation were related to organism productivity and not test substance concentration. No dissolved oxygen concentrations below 60% were observed in the 4 mg/L nominal test substance concentration (high) exposure.
- (8) On days 16 and 19 solution pH's between control and highest test substance exposure concentration deviated by 0.4 standard units or 0.10 units above the allowable 0.3 deviation.
- (9) Test chambers were sealed with transparent plastic sheets rather than teflon-lined closures.
- (10) An opaque sheet was suspended approximately 25 cm above the top of the test chambers to decrease direct light intensity in order to avoid any stress that may have been caused by direct light. The light intensity determined at a later date (2/9/93) under conditions identical to the test conditions was less than 10 ft-c.

- (11) Test organisms were fed a mixture of yeast, Cerophyle and fermented trout chow plus algal suspension rather than blended trout chow plus algal suspension.
- (12) Specific conductivity was measured for control, low, middle, and high test substance concentrations rather than just for controls and high test substance concentrations.
- (13) Test substance stock solution concentration was quantified daily instead of every other day.
- (14) Initial test substance solutions were measured on days 2, 8, 15, and 20.
- (15) Final test substance solutions were measured on days 3, 9, 16, and 21.
- (16) Test substance measurement schedule was also altered to allow for measurement of chambers where 100% of the organisms were affected.
- (17) As the Sponsor was notified, Dr. Dinesh Vaishnav served as a designated QAU staff.

To the best of our current knowledge and scientific understanding these deviations should have no effect on the results presented in this report.

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Physiology. University of Wyoming, Laramie, WY.

# 9.0 PERSONNEL INVOLVED IN STUDY AND THEIR RESPONSIBILITIES

Personnel	Responsibilities
Joe Amato	Study Director
Linda Christensen	Laboratory Assistant
Nancy Jordan	Archivist
Romesh Lakhan	Glassware preparation
Don Mount	Report preparation
Alan Mozol	Sample preparation
David Nessa	Laboratory Assistant
Jo Thompson	Laboratory Assistant
Dinesh Vaishnav	Designated QAU Staff
Minren Xu	Analytical Chemist

Table 1. Isooctyl acrylate (test substance): Immobilization of *D. magna*

Test substance nominal concn (mg/L)	Cumulative number and percentage of immobilized organisms <sup>a</sup>					
	Day 1	Day 2	Day 4	Day 7	Day 14	Day 21
0.0 (control)	0 (0)	0 (0)	0 (0)	1 (3)	1 (3)	2 (5)
0.25	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)	2 (5)
0.5	2 (5)	2 (5)	2 (5)	3 (8)	3 (8)	3 (8)
1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (10)
2	1 (3)	1 (3)	1 (3)	1 (3)	1 (3)	3 (8)
4	0 (0)	1 (3)	5 (13)	18 (45)	27 <sup>b</sup> (68)	36 <sup>b</sup> (90)

<sup>a</sup>Each concentration included four replicate exposures for a total of 40 organisms per concentration. Percent immobilization is in parenthesis.

<sup>b</sup>Value significantly different from the control value at  $p \leq 0.05$ .

Table 2. Insecticide activity (test substance): Number of live and dead young produced, and other related data for 14-day and 21-day intervals

Data on Live Young

Day	Test substance nominal concn (mg/L)																			
	0.0 (control)				0.5				1				2				4			
Rep →	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
7	50	37	0	0	15	48	0	7	19	15	10	33	31	34	48	36	0	0	0	0
8	0	26	10	0	30	9	1	23	19	31	63	33	95	51	39	34	0	13	51	0
9	49	33	85	114	47	16	86	99	31	40	24	45	0	39	0	36	74	14	4	39
10	172	150	10	13	38	125	12	31	73	110	28	99	110	95	170	146	58	40	5	28
11	121	120	107	157	20	24	123	12	56	36	148	31	92	68	31	53	4	17	80	0
12	35	41	127	70	0	7	53	1	2	9	35	26	16	81	0	56	247	88	17	159
13	152	176	1	0	16	192	3	19	183	117	95	151	213	200	212	259	0	69	2	23
14	59	166	198	332	208	3	265	235	88	50	219	83	52	45	31	9	0	7	81	0
14-Day total	638	749	538	686	374	424	543	387	471	408	622	551	609	613	531	619	303	248	240	249
14-Day mean/rep	63.8				47.2				51.3				99.3				200.8			0.3
14-Day mean/original female	66.9				43.2				52.6				59.3				28.0			< 1
15	85	16	217	42	0	37	93	36	3	75	23	112	0	98	0	78	190	54	21	154
16	231	223	0	0	117	344	0	1	88	131	93	160	123	109	205	102	16	124	11	47
17	75	146	141	379	292	25	369	245	179	109	268	104	214	153	103	138	0	30	101	0
18	69	24	238	43	1	19	126	146	76	41	5	113	0	105	0	72	117	18	71	50
19	293	271	15	2	88	427	14	15	112	135	112	164	110	110	180	93	233	168	14	154
20	58	112	109	359	317	5	162	192	252	197	300	161	308	233	269	198	0	80	79	0
21	114	101	291	74	27	50	263	236	113	93	0	94	0	133	0	90	32	2	132	0
21-Day total	1563	1642	1539	1587	1216	1351	1470	1308	1294	1229	1422	1459	1344	1554	1554	1233	1389	979	724	669
21-Day mean/rep	156.3				121.6				129.4				135.3				749.8			< 1
21-Day mean/original female	162.6				133.6				138.6				138.4				74.9			< 1

Table 2. Continued.

Data on Dead Young

Day	Rep →	Test substance nominal concn (mg/L)																																			
		0.0 (control)						0.25						0.5						1						2						4					
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D								
7	3	2	0	0	0	0	3	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
8	0	4	0	0	0	0	0	1	13	8	0	8	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
9	2	3	6	9	8	0	5	5	1	2	3	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
10	4	8	0	0	17	41	0	2	15	16	2	9	5	6	18	14	5	14	3	10	0	0	0	0	0	0	0	0									
11	2	0	0	0	160	1	27	72	33	36	18	12	13	14	7	8	2	17	68	1	7	0	0	0	0	0	0	0									
12	1	0	0	3	5	5	31	8	4	15	17	45	4	12	0	3	34	22	11	24	0	0	0	0	0	0	0	0									
13	76	39	0	0	63	171	0	21	55	110	4	29	38	16	80	28	0	16	0	0	0	0	0	0	0	0	0	0									
14	1	0	0	12	32	0	0	11	1	21	6	4	12	3	2	0	0	7	49	0	0	0	0	0	0	0	0	0									
14-Day total	89	56	6	24	303	221	63	120	124	206	50	107	79	51	107	65	68	143	53	7	0	0	0	0	0	0	0	0									
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	80	1	0	39	0	0	0	0	0	0	0									
16	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	1	5	0	0	0	0	0	0	0	0									
17	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	28	0	0	0	0	0	0	0	0	0									
18	0	0	0	0	0	0	1	0	18	0	0	0	0	0	0	1	6	0	9	17	0	0	0	0	0	0	0	0									
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	1	4	9	3	32	0	0	0	0	0	0									
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	18	6	0	0	0	0	0	0	0	0									
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0									
21-Day total	92	56	6	24	305	221	64	120	135	206	50	109	79	52	129	67	145	171	216	146	7	0	0	0	0	0	0	0	0								

\*The first brood appeared on day 7.

\*The value is significantly lower than the corresponding control value at  $p \leq 0.05$ .

\*Calculated from  $N = 30$ .

Table 3. Isooctyl acrylate (test substance): EC50, IC50, NOEC, and LOEC values (mg/L) for *D. magna*

Test interval	EC50 <sup>a</sup>	IC50	NOEC		LOEC	
			Surv'l <sup>c</sup>	Repro <sup>d</sup>	Surv'l	Repro
14-day (based on initial mean measured concn)	2.93 (2.57-3.33) <sup>b</sup>	1.50 (1.38-1.69)	1.68 <sup>e</sup>	0.79	3.61 <sup>e</sup>	0.19
14-day (based on mean measured concn)	1.99 (1.73-2.29)	0.97 (0.86-1.25)	1.09 <sup>e</sup>	0.51	2.50 <sup>e</sup>	0.11
21-day (based on initial mean measured concn)	2.62 (2.47-2.78)	1.72 (1.58-2.0)	1.79	< 0.20	3.79	0.20
21-day (based on mean measured concn)	1.61 (1.50-1.71)	1.02 (0.95-1.19)	1.06	< 0.13	2.40	0.13

<sup>a</sup>EC50's for days 1, 2, 4, and 7 not calculable due to the lack of immobilization of parental test organisms.

<sup>b</sup>95% confidence intervals.

<sup>c</sup>Surv'l = Survival.

<sup>d</sup>Repro = Reproduction.

<sup>e</sup>Survival values for day 14 not homogeneous, 14-day NOEC and LOEC for survival determined using Steele's Many-One Rank Test. Twenty-one day survival and 14-day and 21-day reproduction values were homogeneous.

Table 4. Isooctyl acrylate (test substance): Mean saturated stock solution concentrations

Test day	Well water concn (mg/L)	Spike recovery %	Stock concn corrected for recovery (mg/L)	Mean stock concn (mg/L)
1	< 0.04*	59.5	11.4, 9.9	10.7
2	< 0.04	87.9	13.2, 12.5	12.8
3	< 0.04	64.4	12.1, 12.0	12.0
4	< 0.04	69.0	9.8, 11.1	10.5
5	< 0.04	101.3	9.8, 11.1	10.5
6	< 0.04	95.7	11.8, 11.6	11.7
7	< 0.04	64.9	13.4, 11.5	12.5
8	< 0.04	40.4	10.8, 13.7	12.3
9	< 0.04	106.5	14.7, 12.7	13.7
10 <sup>b</sup>	< 0.04	99.2	11.0, 10.5	10.7
11 <sup>b</sup>	< 0.04	116.4	9.0, 9.2	9.1
12 <sup>b</sup>	< 0.04	66.0	11.2, 14.1	12.6
13	< 0.04	83.6	11.1, 10.3	10.7
14	< 0.04	105.4	16.4 <sup>c</sup> , 11.9	11.9
15	< 0.04	103.3	10.6, 10.1	10.4
16	< 0.04	127.3	9.9, 9.9	9.9
17	< 0.04	111.9	9.8, 11.0	10.4
18	< 0.04	85.7	13.6, 13.8	13.7
19	< 0.04	92.3	10.4, 9.1	9.7
20	< 0.04	114.1	10.2, 8.9	9.6
21	< 0.04	102.7	9.8, 10.5	10.1

\*The analytical method detection limit was < 0.04 mg/L.

<sup>b</sup>Sample extracts were analyzed three days later due to mass spectrometer malfunction. The extracts were stored in a freezer until analyzed.

The value was rejected because it was unreasonably high. The sample may have contained undissolved test substance.

Table 5. Isooctyl acrylate (test substance): Matrix spike recoveries

Matrix	Day of analysis	Test substance concn (mg/L)		% Recovery
		Target	Measured	
Deionized water	2	0.0	< 0.04 <sup>a</sup>	NC <sup>b</sup>
	3	0.0	< 0.04	NC
	8	0.0	< 0.04	NC
	9	0.0	< 0.04	NC
	15	0.0	< 0.04	NC
	20	0.0	< 0.04	NC
	21	0.0	< 0.04	NC
Deionized water spike	2	0.44	0.36	81.1
	3	0.44	0.44	100.4
	8	0.13 <sup>a</sup>	0.10	72.8 <sup>d</sup>
	9	0.44	0.45	102.6 <sup>d</sup>
	15	0.44	0.36	82.6
	16	0.44	0.45	101.1
	20	0.44	0.35	79.9
	21	0.44	0.33	75.4
Mean spike recovery 87 ± 10.1 %				

Table 5 continued on the next page.

Table 5. Continued.

Matrix	Day of analysis	Test substance concn (mg/L)		% Recovery
		Target	Measured	
Well water blank (no food) Initial test solution	2	0.0	< 0.04*	NC <sup>b</sup>
	8	0.0	< 0.04	NC
	15	0.0	< 0.04	NC
	20	0.0	< 0.04	NC
Well water spike (no food) Initial test solution	2	0.44	0.38	85.9
	8	0.13 <sup>c</sup>	0.11	85.6 <sup>d</sup>
	15	0.44	0.42	95.1
	20	0.44	0.34	77.7
Mean spike recovery 86 ± 7.1 %				
Well water blank (food) Final test solution	3	0.0	< 0.04*	NC <sup>b</sup>
	9	0.0	< 0.04	NC
	16	0.0	< 0.04	NC
	21	0.0	< 0.04	NC
Well water spike (food) Final test solution	3	0.44	0.26	58.7
	9	0.44	0.49	110.7 <sup>d</sup>
	16	0.44	0.46	104.0
	21	0.44	0.36	78.7
Mean spike recovery 81 ± 18.5 %				

\*Method detection limit was 0.04 mg/L test substance.

<sup>b</sup>NC - not calculated.

<sup>c</sup>Target was erroneously spiked at 0.132 mg/L.

<sup>d</sup>Values excluded from the calculation of the mean spike recovery.

Table 6. Isooctyl acrylate (test substance): Initial test substance concentrations corrected for recoveries

Test substance nominal concn (mg/L)	Initial measured test substance concn (mg/L) corrected for recovery <sup>a</sup>				Mean Initial Concn $\pm$ SD <sup>b</sup>
	Day 2	Day 8	Day 15	Day 20	
0.0 (control)	0	0	0	0	0
0.25	0.19	0.02	0.14	0.27	0.20 $\pm$ 0.666
0.5	0.42	0.04	0.36	0.64	0.47 $\pm$ 0.151
1	0.79	0.08	0.80	0.96	0.85 $\pm$ 0.095
2	1.68	0.24	1.56	2.13	1.79 $\pm$ 0.301
4	3.61	1.04	3.00	4.76	3.79 $\pm$ 0.694

<sup>a</sup>Concentrations corrected for the test substance recovery (listed in Table 5) from well water spike for that day.

<sup>b</sup>Day 8 values excluded from the calculation of the mean and standard deviation. The values obtained for day 8 were low and appeared to be incorrect as the final test substance measurements from these solutions (day 9) were comparable to the final values obtained on days 3, 16, and 21.

Table 7. Isooctyl acrylate (test substance): Final test substance concentrations (mg/L) corrected for recoveries

Test Substance nominal concn (mg/L)	Rep	Final measured test substance concn (mg/L) corrected for recovery				Mean final concn $\pm$ SE
		Day 3	Day 9	Day 16	Day 21	
0.0 (control)	A	< 0.04	< 0.04	< 0.04	< 0.04	
	B	< 0.04	< 0.04	< 0.04	< 0.04	
	C	< 0.04	< 0.04	< 0.04	< 0.04	
	D	< 0.04	< 0.04	< 0.04	< 0.04	
	Mean	< 0.04	< 0.04	< 0.04	< 0.04	
0.25	A	0.04	0.05	0.06	0.11	
	B	0.10	0.09	0.05	0.09	
	C	< 0.04	< 0.04	0.07	< 0.04	
	D	< 0.04	0.07	0.06	0.07	
	Mean	0.34	0.05	0.06	0.07	0.06 $\pm$ 0.013
0.5	A	0.12	0.07	0.08	0.10	
	B	0.19	0.10	0.08	0.09	
	C	0.10	0.11	0.05	0.06	
	D	0.11	0.10	0.09	0.10	
	Mean	0.13	0.10	0.08	0.09	0.10 $\pm$ 0.022
1	A	0.20	0.15	0.18	0.14	
	B	0.22	0.17	0.16	0.10	
	C	0.24	0.25	0.15	0.11	
	D	0.23	0.13	0.20	0.09	
	Mean	0.22	0.18	0.17	0.11	0.17 $\pm$ 0.055

Table 7 continued on the next page.

Table 7. Continued.

Test Substance nominal concn (mg/L)	Rep	Final measured test substance concn (mg/L) corrected for recovery <sup>a</sup>				Mean final concn $\pm$ SE <sup>b</sup>
		Day 3	Day 9	Day 16	Day 21	
2	A	0.74	0.40	0.37	0.13	
	B	0.39	0.34	0.28	0.17	
	C	0.35	0.59	0.39	0.13	
	D	0.47	0.36	0.34	0.17	
	Mean	0.49	0.42	0.35	0.14	
4	A	1.37	0.27	1.11	0.60	
	B	1.08	0.86	1.17	0.61	
	C	1.63	0.87	0.89	0.56	
	D	1.49	0.93	0.74	0.56	
	Mean	1.39	0.73	1.02	0.59	

<sup>a</sup>Concentrations corrected for test substance recovery (listed in Table 5) from fed well water spike for that day.

<sup>b</sup>Day 9 values are excluded from mean final concentrations and standard error calculations. The values obtained for day 8 were low and appeared to be incorrect as the final test substance measurements from these solutions (day 9) were comparable to the final values obtained on days 3, 16, and 21. Although the day 9 values appear to be correct, they were also excluded from calculating the mean concentrations so that the mean concentrations are not skewed toward the final concentration values.

<sup>c</sup>Value is duplicate of replicate 4C.

<sup>d</sup>All organisms exposed in replicate 4C died before day 21.

Table 8. Isooctyl acrylate (test substance); Mean test substance concentrations for 14-day and 21-day effect determinations

Test substance nominal concn (mg/L)	Day 14		Day 21	
	Initial concn <sup>a</sup> (mg/L)	Mean initial and final concn for days 2 & 3 (mg/L) <sup>b</sup>	Mean initial concn (mg/L)	Mean initial and final concn (mg/L) <sup>c</sup>
0.0 (control)	0	0	0	0
0.25	0.19	0.11	0.20	0.13
0.5	0.42	0.28	0.47	0.29
1	0.79	0.51	0.85	0.51
2	1.68	1.09	1.79	1.06
4	3.41	2.50	3.79	2.40

<sup>a</sup>Values from day 2 initial measurements only.

<sup>b</sup>Mean values from day 2 initial and day 3 final measurements.

<sup>c</sup>Values exclude day 8 initial measurements.

<sup>d</sup>Values exclude day 8 initial and day 9 final measurements.

Table 9. Isooctyl acrylate (test substance): Hardness (as CaCO<sub>3</sub>, mg/L) of control and high test substance exposures

Test day	Test substance nominal concn (mg/L)	
	0.0 (control)	4
1	169	169
2	160	167
3	131	132
4	152	135
5	134	138
6	136	133
7	140	140
8	138	130
9	132	131
10	134	131
11	128	127
12	133	133
13	131	157
14	164	164
15	170	169
16	168	166
17	168	167
18	165	167
19	166	166
20	165	165
21	168	162
Mean $\pm$ SD	150 $\pm$ 16.6	150 $\pm$ 16.7
Range	128 - 170	127 - 167

Table 10. Isooctyl acrylate (test substance): Alkalinity (as CaCO<sub>3</sub>, mg/L) of control and high test substance exposures

Test day	Test substance nominal concn (mg/L)	
	0.0 (control)	4
1	143	142
2	134	140
3	109	108
4	105	108
5	119	116
6	115	114
7	112	113
8	110	109
9	109	109
10	107	108
11	107	108
12	109	109
13	109	132
14	147	148
15	147	147
16	147	149
17	148	149
18	148	148
19	151	149
20	149	149
21	148	147
Mean $\pm$ SD	127 $\pm$ 19.0	129 $\pm$ 18.6
Range	105 - 151	108 - 149

Table 11. Isooctyl acrylate (test substance): Temperatures (°C) of monitoring chamber solutions

Test day	Test substance nominal concn (mg/L)							
	0.0 (control)		0.25		1		4	
	A*	B*	A	B	A	B	A	B
1	20.8	20.5	20.8	20.5	20.8	20.5	20.8	20.5
2	20.5	21.0	20.5	21.0	20.5	21.0	21.2	21.4
3	21.2	21.2	21.2	21.2	21.2	21.4	21.2	21.4
4	21.6	21.3	21.4	21.3	21.4	21.3	21.4	21.3
5	21.2	20.8	21.2	20.8	21.8	20.8	21.8	20.8
6	21.0	20.6	20.8	20.6	20.8	20.8	21.0	20.8
7	20.6	20.4	20.6	20.4	20.6	20.4	20.6	20.4
8	20.6	21.6	20.6	21.6	20.6	21.6	20.8	21.6
9	21.2	21.6	21.2	21.6	21.2	21.4	21.2	21.6
10	21.6	21.2	21.6	21.2	21.6	21.4	21.6	21.8
11	21.6	22.4	21.6	22.4	21.6	22.4	21.6	22.4
12	22.0	20.8	22.0	21.0	22.0	21.0	22.0	21.0
13	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
14	20.8	21.0	20.8	21.0	20.8	21.0	20.8	21.0
15	21.0	20.8	21.0	20.8	20.7	20.8	20.6	20.8
16	20.2	21.2	20.2	21.2	20.2	21.2	20.2	21.2
17	21.6	21.0	21.4	21.4	21.4	21.0	21.3	21.0
18	21.4	21.5	21.4	21.5	21.3	21.4	21.2	21.5
19	21.4	20.8	21.4	20.8	21.4	21.0	21.4	20.9
20	20.8	21.2	20.8	21.2	20.8	21.0	20.8	21.2
21	20.2	21.0	20.2	21.0	20.3	21.0	20.4	21.0
Mean	21.1	21.1	21.0	21.1	21.0	21.1	21.1	21.2
± SD	± 0.5	± 0.4	± 0.5	± 0.4	± 0.5	± 0.4	± 0.5	± 0.5
Range	20.2 - 22.0	20.4 - 22.4	20.2 - 22.0	20.4 - 22.4	20.2 - 22.0	20.4 - 22.4	20.2 - 22.0	20.4 - 22.4

\*A = Initial measurements.

\*B = Final measurements.

Table 12. Isooctyl acrylate (test substance): DO concentration (mg/L) of monitoring chamber solutions

Test day	Test substance nominal concn (mg/L)							
	0.0 (control)		0.25		1		4	
	A <sup>a</sup>	B <sup>a</sup>	A	B	A	B	A	B
1	7.4	7.9	7.4	8.2	7.3	7.5	7.4	7.1
2	9.0	7.4	8.8	8.2	8.7	7.5	8.7	6.1
3	8.0	6.3	8.4	6.2	8.2	6.2	8.3	6.0
4	8.0	6.3	8.3	6.0	8.4	5.5	8.3	5.0
5	8.0	6.1	8.6	6.1	8.7	5.2	8.6	5.3
6	8.2	6.1	8.5	6.1	8.6	6.4	8.7	6.4
7	8.3	6.0	8.4	6.1	8.5	5.2	8.6	8.3
8	8.6	7.5	8.6	6.8	8.8	5.2	8.8	6.3
9	8.0	5.3	7.9	5.8	8.0	6.1	8.2	5.8
10	8.1	6.7	8.1	5.3	8.1	5.4	8.2	5.6
11	7.8	6.2	8.0	5.4	8.0	5.4	8.0	5.5
12	7.5	5.8	7.5	8.0	7.6	8.0	7.6	8.7
13	8.5	6.5	8.4	6.0	8.3	6.8	8.4	8.4
14	8.2	7.4	8.3	6.2	8.2	5.6	8.1	7.6
15	8.0	6.1	8.2	6.5	8.1	5.5	8.3	7.0
16	8.4	5.2	8.5	5.3	8.5	6.3	8.5	7.4
17	8.4	5.4	8.5	5.4	8.6	5.6	8.6	6.2
18	7.9	5.3	8.1	5.1	8.1	5.1	8.2	6.1
19	8.5	4.8	8.5	4.9	8.6	5.9	8.6	6.5
20	8.1	5.6	8.2	5.2	8.2	6.2	8.2	6.4
21	8.2	4.5	8.2	5.2	8.4	4.8	8.4	5.7
Mean ± SD	8.1 ± 0.4	6.1 ± 0.9	8.2 ± 0.3	6.1 ± 1.0	8.3 ± 0.4	6.0 ± 0.9	8.3 ± 0.4	6.5 ± 1.0
Range	7.4 - 9.0	4.5 - 7.9	7.4 - 8.8	4.9 - 8.2	7.3 - 8.8	4.8 - 6.0	7.4 - 8.7	5.0 - 8.7

<sup>a</sup>A = Initial measurements.

<sup>a</sup>B = Final measurements.

Table 13. Isooctyl acrylate (test substance): pH of monitoring chamber solutions

Test day	Test substance nominal concn (mg/L)							
	0.0 (control)		0.25		1		4	
	A <sup>a</sup>	B <sup>b</sup>	A	B	A	B	A	B
1	7.9	7.8	7.9	8.0	7.9	7.9	7.9	7.8
2	8.0	8.1	7.7	8.0	7.8	8.0	7.8	7.8
3	8.2	7.8	8.3	7.9	8.3	7.8	8.3	7.8
4	8.2	7.8	8.2	7.8	8.2	7.8	8.2	7.7
5	8.2	7.8	8.2	7.8	8.2	7.8	8.2	7.7
6	8.2	7.7	8.2	7.7	8.2	7.7	8.2	7.7
7	8.2	7.8	8.2	7.8	8.2	7.8	8.2	7.8
8	8.4	7.7	8.4	7.7	8.4	7.7	8.4	7.8
9	8.4	7.7	8.4	7.7	8.4	7.7	8.4	7.8
10	8.4	7.6	8.4	7.6	8.4	7.6	8.4	7.8
11	8.3	7.7	8.4	7.8	8.4	7.7	8.4	7.8
12	8.4	7.8	8.4	7.7	8.4	7.7	8.4	7.8
13	8.4	7.7	8.4	7.7	8.4	7.7	8.5	7.9
14	8.5	7.7	8.5	7.8	8.5	7.9	8.5	8.0
15	8.4	7.9	8.4	7.9	8.4	7.9	8.4	8.2
16	8.5	7.7	8.5	7.8	8.5	7.9	8.5	8.1
17	8.5	7.8	8.5	7.8	8.5	7.8	8.5	8.0
18	8.5	7.8	8.5	7.8	8.5	7.7	8.5	8.0
19	8.5	7.7	8.5	7.8	8.5	7.8	8.5	8.1
20	8.5	7.8	8.5	7.8	8.5	7.7	8.5	8.0
21	8.6	7.8	8.6	7.8	8.5	7.7	8.5	7.9
Range	7.9 - 8.6	7.6 - 8.1	7.7 - 8.6	7.6 - 8.0	7.8 - 8.5	7.6 - 8.0	7.8 - 8.5	7.7 - 8.2

<sup>a</sup>A = Initial measurements

<sup>b</sup>B = Final measurements

Table 14. Isooctyl acrylate (test substance): Conductivities ( $\mu\text{mhos/cm}$ ) of monitoring chamber solutions

Test day	Test substance nominal concn (mg/L)			
	0.0 (control)	0.25	1	4
1	375	381	379	377
2	363	377	378	374
3	305	310	313	312
4	314	312	314	318
5	315	309	322	323
6	330	322	321	320
7	313	314	315	317
8	285	285	285	286
9	301	288	389	293
10	282	283	286	287
11	280	282	285	287
12	287	290	295	294
13	296	305	303	325
14	308	325	326	329
15	345	347	344	345
16	338	342	343	344
17	311	319	325	321
18	321	327	330	331
19	334	335	340	341
20	321	324	325	326
21	320	325	333	334
Mean $\pm$ SD	316 $\pm$ 25	319 $\pm$ 27	321 $\pm$ 27	323 $\pm$ 25
Range	280 - 375	282 - 381	285 - 379	286 - 377

Table 15. Isooctyl acrylate (test substance): QA criteria and test acceptability

QA criterion	Results
Less than 20% cumulative mortality must occur for dilution water control organisms.	Five percent cumulative mortality occurred in the dilution water control exposure.
Dissolved oxygen concentration must be maintained at a minimum of 60% air saturation at the test temperature.	Control exposures had 3 DO measurements below 60% - 51%, 54%, and 58%. Low test substance concentrations had 4 DO measurements below 60% - 58%, 58%, 55%, and 58%. Middle test substance concentrations had 5 DO measurements below 60% - 54%, 58%, 59%, 58%, and 58%.
pH in dilution water controls and highest test concentration must not deviate by not more than 0.3 standard units.	On two exposure days, final pH deviated between the control exposure and high test concentration exposure by 0.4 standard units.
First brood must be released before or on day 9.	First brood was released on day 7.
Test organisms in dilution water must produce a minimum of 20 young per surviving female at 14 days and, 60 young per surviving female at 21 days.	Mean young production per surviving female exposed to the dilution water control was 69 at 14 days and 171 at 21 days.
Test duration must be 21 days.	Test duration was 21 days.

Abel Corporation/Abel-Debut  
Environmental Testing Division  
Abel Report ED/ 000-DMC-23M  
Abel Study ED/ 3000-000-01

#### Appendix A

Chemical analyses of well water and trout chow

# Chemical Analysis of Well Water<sup>a</sup>

ASCI Corporation/ASCI-Duluth  
Environmental Testing Division  
ASCI Report ID# 888-DMC.1334  
ASCI Study ID# 5080-009-00

Parameter	µg/L	MDL <sup>b</sup> (µg/L)	Parameter	µg/L	MDL <sup>b</sup> (µg/L)	Parameter	Unit	Conc.
Aldrin	ND <sup>c</sup>	0.3	Naled	ND	2.5	Total Suspended Solids	mg/L	< 4
A-BHC	ND	3.0	Diazinon	ND	1.0	Ammonia Nitrogen	mg/L	< 0.05
B-BHC	ND	0.4	Rotenone	ND	0.5	Total Kjeldahl Nitrogen	mg/L	0.18
D-BHC	ND	4.0	Chlorpyrifos	ND	0.5	Chemical Oxygen Demand	mg/L	9
Chlordane (Gamma)	ND	1.0	DEF	ND	0.5	Total Cystate	mg/L	< 0.01
Chlordane (Alpha)	ND	1.0	Polstar	ND	0.5	Aluminum	µg/L	< 100
4,4'-DDD	ND	0.3	Phosalone	ND	0.5	Arsenic	µg/L	< 2
4,4'-DDE	ND	0.3	Orthene	ND	5.0	Cadmium	µg/L	< 0.5
4,4'-DDT	ND	0.3	Coumaphos	ND	5.0	Calcium	mg/L	46.5
Dieldrin	ND	0.3	Dichlorvos	ND	1.0	Cobalt	µg/L	< 2
Endosulfan I	ND	1.0	Mevinphos	ND	3.5	Chromium	µg/L	< 2
Endosulfan II	ND	1.0	Trifluralin	ND	0.5	Copper	µg/L	13
Endosulfan Sulfate	ND	1.0	Ethoprop	ND	0.5	Iron	µg/L	3
Endrin	ND	1.0	Phorate	ND	0.5	Lead	µg/L	< 2
Endrin Aldehyde	ND	0.3	Diazotolone	ND	0.5	Magnesium	mg/L	16.3
Heptachlor	ND	0.05	Methyl Parathion	ND	0.5	Mercury	µg/L	< 0.3
Heptachlor Epoxide	ND	0.3	Morphos	ND	0.5	Nickel	µg/L	< 2
Lindane (G-BHC)	ND	0.1	Fenitrothion	ND	0.5	Potassium	mg/L	< 0.5
Toxaphene	ND	2.0	Diphosamid	ND	0.5	Selenium	µg/L	< 1
Methoxychlor	ND	1.0	Ethion	ND	0.5	Silver	µg/L	< 1
Endrin Ketone	ND	1.0	Fenvalerate	ND	1.0	Sodium	mg/L	6.5
PCB 1016	ND	1.0	Carbophenanthion	ND	1.0	Zinc	µg/L	30
PCB 1221	ND	1.0	Diazinon	ND	0.5	Notes: Ca/Mg = 2.85 and Na/K = > 13		
PCB 1232	ND	1.0	Dimethoate	ND	0.5			
PCB 1242	ND	1.0	Malathion	ND	2.0			
PCB 1248	ND	1.0	Parathion	ND	0.5			
PCB 1254	ND	1.0	Methyl Trithion	ND	1.0			
PCB 1260	ND	1.0	Proxion	ND	0.5			
			Trichloromet	ND	0.5			

<sup>a</sup> The Water Was Collected from Two Hatches (Massena) Area and Analyzed During August-September 1991

<sup>b</sup> MDL = Method Detection Limit

<sup>c</sup> ND = Not Detected

# Chemical Analysis of Trout Chow<sup>a</sup>

ASel Corporation/ASel-Duluth  
Environmental Testing Division  
ASel Report ID# 003-DMC-RSM  
ASel Study ID# 5030-003-08

Parameter	Trout Chow 1/8" Pellets (µg/kg)	MDL <sup>b</sup> (µg/kg)
Aldrin	ND <sup>c</sup>	16
A-BHC	ND	16
B-BHC	ND	16
D-BHC	ND	16
Chlordane (Gamma)	ND	16
Chlordane (Alpha)	ND	80
4,4'DDD	ND	16
4,4'DDE	ND	16
4,4'DDT	ND	16
Dieldrin	ND	16
Endosulfan I	ND	16
Endosulfan II	ND	16
Endosulfan Sulfate	ND	16
Endrin	ND	16
Endrin Aldehyde	ND	16
Heptachlor	ND	16
Heptachlor Epoxide	ND	16
Lindane (G-BHC)	ND	16
Toxaphene	ND	160
Methoxychlor	ND	32
Endrin Ketone	ND	16
PCB 1016	ND	160
PCB 1221	ND	160
PCB 1232	ND	160
PCB 1242	ND	160
PCB 1248	ND	160
PCB 1254	ND	160
PCB 1260	ND	160

<sup>a</sup> The Trout Chow Sample Was Collected at Testing Facility and Analyzed During August-September 1991

<sup>b</sup> MDL = Method Detection Limit

<sup>c</sup> ND = Not Detected

Asel Corporation/Asel-Dubois  
Environmental Testing Division  
Asel Report ID# 003-DMA-C.234d  
Asel Study ID# 5050-005-08

#### Appendix B

Isocetyl acrylate: Method validation for analysis from water

**STUDY TITLE**

ISOOCTYL ACRYLATE: METHOD VALIDATION FOR ANALYSIS FROM WATER

**AUTHORS**

Minren Xu and Dinesh Vaishnav

**STUDY COMPLETED**

May 28, 1992

**TESTING FACILITY**

ASCI Corporation  
ASCI-Duluth Environmental Testing Division  
112 East Second Street  
Duluth, MN 55805

Tel. No. (218) 722-4040

**STUDY IDENTIFICATION NUMBERS**

ASCI Study ID# 5030-003-01

3M Company Study ID# J2774

**CERTIFIED COPY**

Signature: [Signature] Date: 3/26/92

Page 1 of 27

# CERTIFICATION OF GOOD LABORATORY PRACTICE COMPLIANCE

To the best of my knowledge, this study was conducted in accordance with OECD Good Laboratory Practice Standards (OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice 1981).

Study Director: mx Date: 3/26/93  
Minren Xu  
ASCI Corporation/ASCI-Duluth  
Environmental Testing Division

Based on the signatures of the Study Director and the Quality Assurance Auditor, this study, to the best of our knowledge, was conducted in accordance with OECD Good Laboratory Practice Standards (OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice 1981).

Sponsor: \_\_\_\_\_ Date: \_\_\_\_\_  
Submitter: \_\_\_\_\_ Date: \_\_\_\_\_

### STATEMENT OF QUALITY ASSURANCE

The study data were reviewed by the ASOI-Duluth Environmental Testing Division Quality Assurance Unit to assure that standard operating procedures and guidelines used to conduct this study were followed, and this report is an accurate reflection of the raw data. The types of audits performed are listed in the following table.

Type of Audit for ASOI Study ID# 5030-003-01	Audit Date	Date Reported to Study Director and Management
Study Plan	12-17-1991	12-17-1991
In-Life Phase	12-19-1991	12-19-1991
Raw Data and Draft Report	01-09-1992	01-09-1992
Final Report	05-28-1992	05-28-1992

Alan Mozol  
Acting Manager, Quality Assurance Unit

Date: \_\_\_\_\_

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STUDY SUMMARY TABLE

Study Title	Isooctyl Acrylate: Method Validation for Analysis from Water
Good Laboratory Practice Standards	As promulgated under the OECD Council Decision C(81)30, Annex 2: OECD Principles of Good Laboratory Practice (OECD 1981).
Sponsor	Rich Purdy, 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106; Tel No. (612) 778-5379.
Sponsor's Representative	Susan A. Beach, 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106; Tel No. (612) 778-7452.
Testing Facility	ASCI Corporation/ASCI-Duluth Environmental Testing Division, 112 East Second Street, Duluth, MN 55805; Tel. No. (218) 722-4040.
Study Director	Minren Xu
Acting QAU Manager	Alan Mozol
Testing Facility Director	Donald Mount
Study Initiation Date	December 17, 1991
Test Dates	December 17-19, 1991
Test Substance	Isooctyl acrylate (CAS No. 29590-42-9, MC-857, Lot 3290), 99.75% acrylate (as determined by Sponsor NB# 92391), liquid.

Test Description	Calibration Curves: (1) Standard solutions of various test substance concentrations and reagent (acetone) blank were prepared in acetone, (2) all solutions and reagent blank were analyzed twice by GC/MS, and (3) data were used to calculate regression equations, analytical method detection limits and other statistics.
Test Description (continued)	Spike Solutions and Recoveries: (1) Three replicates of test substance low and high spike solutions, and method blank (deionized water) were prepared using deionized water, (2) spike solutions and method blank were extracted using solid/liquid extraction technique, and extracts analyzed by GC/MS, and (4) data were used to calculate test substance recoveries from spike solutions.

<p>Test Results</p>	<p>Percentage relative standard deviation (% RSD): First calibration curve -- 0.81% Second calibration curve -- 1.93%</p> <p>Correlation coefficient (r): First calibration curve -- 1.000 Second calibration curve -- 0.999</p> <p>Method detection limit (MDL): With first calibration curve -- 0.04 mg/L With second calibration curve -- 0.04 mg/L</p> <p>Mean percentage recovery (R) from low spike solution (0.123 mg/L test substance): 85.91%</p> <p>Mean percentage recovery (R) from high spike solution (8.8 mg/L test substance): 103.48%</p> <p>Combined mean percentage (R) recovery from low and high spike solutions: 94.70%</p>
<p>Location of Raw Data and Final Report</p>	<p>ASCI Corporation/ASCI-Duluth Environmental Testing Division, 112 East Second Street, Duluth, MN 55805; Tel. No. (218) 722-4040.</p>

## 1.0 INTRODUCTION

The test substance, isooctyl acrylate, is an ester made from primarily isooctanol and acrylic acid. According to OECD recommendations for new chemical substances (OECD Council Decision, 12th May, 1981; C(81)30), (1) the test substance physical-chemical properties and toxicities to various aquatic organisms need to be determined, and (2) chemical effects must be reported on the basis of measured chemical concentration. For the latter, there was a need to validate an analytical method so that test substance concentration can be determined from matrices employed in various tests. The analytical method was provided by the Sponsor.

The objectives of the present study were: (1) to develop an acceptable calibration curve, (2) to calculate detection limit of the analytical method, and (3) to determine test substance recoveries from spike solutions prepared using deionized water.

## 2.0 TEST METHODS

2.1 Formulas and Definitions. The formulas and definitions used in this study were:

- (1) Test Substance Mean Percentage Recovery (R)

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Environmental Testing Division  
ASCI Report ID# 000-METH.33M  
ASCI Study ID# 5030-003-01

$$R_i = (\text{Measured concentration} / \text{Target concentration}) \times 100$$

The mean R was calculated using individual  $R_i$  values which fell within  $R \pm 3SD$  range. If, the mean R was not between 80% and 120%, all measured concentrations were corrected accordingly.

(2) Method Detection Limit (MDL)

MDL = 3 X background signal in reagent blank

(3) Relative Standard Deviation of Calibration Curve (% RSD)

$$\% \text{ RSD} = (\text{Standard deviation of slope/slope}) \times 100$$

- (4) The sample response was corrected for the response of the method blank, if interference from the method blank was expected to have any effect on the sample response.

2.2 Test Substance. The test substance, isooctyl acrylate, (CAS No. 29590-42-9, [ ] Lot 3290) was received at ASCI on October 3, 1991 in one amber glass bottle placed in a sealed metal container. The test substance was stored at room temperature as received. According to a material safety data sheet and a written communication provided by the Sponsor, (1) the test substance was a clear, colorless, mobile liquid with acrylate odor, (2) the test substance concentration in deionized water can be analyzed by a GC method, (3) the test substance was 99.75% acrylate as determined by Sponsor NB# 92391 and (4) the test substance had 1 mm Hg vapor

pressure at 50°C. The Sponsor also had information that based on the chemical structure, there would be essentially no dissociation or pH-dependent hydrolysis of the test substance at environmental pH levels.

2.3 Apparatus and Reagents. The apparatus and reagents used were:

- (1) HP model 5890 gas chromatograph with 30 m 0.32 DB-5 (J & W Scientific) capillary column;
- (2) HP model 5970 mass spectrometer;
- (3) Pesticide grade methylene chloride and other solvents;
- (4) Deionized water; and
- (5) Extraction apparatus.

2.4 GC/MS Analysis. The analytical conditions were:

- (1) Carrier gas: Helium at a total inlet purge flow of 40 ml/minute and a septum purge flow of 1 ml/minute with splitless injection mode;
- (2) Temperature program: Isothermal at 70°C for 2 minutes then 8°C per minute to 200°C;
- (3) Ionization source: Electron impact with a scan range of 20-500 mμ; and
- (4) Detection method: Total ion chromatograph.

Before analysis, mass spectrometer was tuned using autotune program. A GC column performance test was conducted using column check sample (HP Sample A) to meet the criteria recommended by the manufacturer. A post GC/MS performance test was carried out by running a column check sample (HP Sample A) to ensure the stability of the instrument during the analytical test.

2.5 Calibration Curve. Two test substance stock solutions were prepared in acetone in 10-ml volumetric flasks. The first solution contained 1,760 mg/L test substance and the second solution contained 880 mg/L test substance. Subsequently, four standard solutions were prepared by adding appropriate volumes of the second stock solution to 10-ml volumetric flasks and diluting to volume with acetone. A reagent blank was prepared using acetone.

Each stock and standard solution, and reagent blank were analyzed twice by GC/MS. The instrument responses, except of reagent blank, from 8.95 to 12.958 minutes were integrated using a group integration method, and correlated with the test substance nominal concentration. The relative standard deviations of calibration curves (% RSD) and method detection limits (MDL) were then calculated.

**2.6 Spike Solutions.** Three replicates of a low level spike solution were prepared by adding 7  $\mu$ l of test substance second stock solution (880 mg/L) to 50 ml of deionized water. This produced a target spike concentration of 0.123 mg/L test substance. Similarly, three replicates of a high level spike solution were prepared by adding 5 ml of test substance second stock solution (880 mg/L) to 500 ml of deionized water. This produced a target spike concentration of 8.8 mg/L test substance. A method blank was prepared using 500 ml of deionized water.

**2.7 Test substance Extraction and Analysis.** Both spike solutions and method blank were first extracted, using solid/liquid extraction procedure, and extracts analyzed by GC/MS. The extraction procedure was:

- (1) Placed a 25-mm (with 50 ml sample) or 47-mm diameter (with > 50 ml sample) Empore<sup>™</sup> extraction disk (J.T. Baker, Inc.) between a filter base and reservoir;
  - (2) Pre-washed the disk with 10 ml of methylene chloride (elution solvent);
  - (3) Applied vacuum to draw the solvent through the disk;
  - (4) Added 10 ml of methanol, applied vacuum and left a meniscus of methanol just above the top of the disk
- (NOTES: RELEASED VACUUM BEFORE THE DISK WAS DRY. DID NOT

ALLOW DISK TO DRY AT ANY TIME BEFORE SAMPLE FILTRATION WAS COMPLETED);

- (5) Added 20 ml of deionized water to the reservoir, applied vacuum and left a meniscus of water just above the top of the disk;
- (6) Added 5 ml methanol per liter of sample and mixed well;
- (7) Poured sample into the reservoir and applied vacuum. The minimum filtration time was 10 minutes/L of sample;
- (8) After the sample was processed, drew air through disk for 15 minutes;
- (9) Placed the tip of the filter base into a test tube inside the filtration flask;
- (10) Rinsed the volumetric flask with 2.5 ml (with 50 ml sample) or 4-5 ml (with > 50 sample) methylene chloride and added the solvent to the reservoir;
- (11) Drew half the solvent through the disk and let stand for approximately 1 minute. Drew the remainder through the disk;
- (12) Repeated Steps 10 and 11 three times;
- (13) Collected a measured volume of methylene chloride extract; and
- (14) Processed the method blank in the same way (Steps 1 to 13) as the sample.

For low spike solutions, extracts were first concentrated under a gentle stream of nitrogen gas and the volumes of concentrated extracts measured. The extracts of both low and high spike solutions were then transferred to analytical vials and analyzed for the test substance concentrations using the GC/MS instrument. The instrument was operated as per manufacturer's recommendation.

2.8 Test Substance Recovery. The instrument responses between 8.95 and 12.958 minutes were integrated using a group integration method, and fitted to the first calibration curve to determine test substance concentrations. These data were then used to calculate the test substance percentage recoveries from spike solutions.

2.9 Test Substance Analysis During Various Tests. Several physical/chemical and toxicity tests were performed separately with this test substance. In analyzing the test substance concentrations in aqueous samples from these tests, the following procedure was used:

- (1) At each test initiation, developed an acceptable new calibration curve with a relative standard deviation (% RSD) within 10%;

- (2) Each day when test substance concentrations in aqueous samples from a particular test were analyzed, re-validated the previous calibration curve (from Step 1) using at least two standard solutions, or developed a new acceptable calibration curve with a relative standard deviation (% RSD) within 10%. In case of re-validation, the previous calibration curve was considered valid and the same regression equation (From Step 1) was used, if the measured and nominal concentrations of standard solutions did not differ by more than 10%;
- (3) Each time when test substance concentrations in aqueous samples from a particular test were analyzed, standard (deionized water) and test (e.g. well water, algal medium etc.) matrices blanks, and spiked standard and test matrices were prepared. The test substance spike concentration was close to the lowest nominal concentration used in a particular test. Generally, the spike concentrations were similar to the low spike concentration (0.123 mg/L) used in this method validation study;
- (4) Analyzed both standard and test matrices and calculated percentage spike recoveries;

- (5) Accepted spike recoveries if they were within the same range ( $85.91 \pm 22.859\%$ ) as low spike recovery established from this method validation study;
- (6) Each time when test substance concentrations in aqueous samples from a particular test were analyzed, corrected (to 100%) test substance concentrations in aqueous samples for the percentage matrix spike recovery for that time.

2.10 Data Analysis. All data were analyzed using Minitab<sup>®</sup> statistical software (Minitab, Inc. 1988), MS ChemStation software (HP 1990) which interfaced the GC/MS instrument, and a scientific calculator.

### 3.0 RESULTS

Six test substance solutions, including two stock and four standard solutions (Table 1), were used to prepare two calibration curves. The use of a broad range of solution concentrations was important because the test substance concentrations in biological tests are expected to range from approximately 0.1 mg/L to the test substance water solubility concentration (12.44 mg/L).

The samples from physical/chemical and biological tests will be extracted and test substance concentrations eluted in approximately 15 ml of solvent (actual extract volume will be measured). Accordingly, one solution (standard solution 1) used for the two calibration curves had a test substance concentration approximately 3 fold greater than the method detection limit (MDL) of 0.04 mg/L (Table 1). All other solutions, except the first stock solution, were below and near the test substance solubility (12.44 mg/L) in deionized water (Table 1). The test substance concentration in the first stock solution was approximately twice the solubility concentration.

The GC/MS responses in two calibration curves are listed in Table 2. Correlations of GC/MS response (ordinate) and test substance nominal concentration (abscissa) had correlation coefficients (r) of 1.000 and 0.999 for the first and second calibration curves, respectively (Table 3). The slopes from both curves differed by approximately 0.32%, and relative standard deviations (% RSD) of slopes were 0.81% and 1.93% for the first and second calibration curves, respectively (Table 3). The detection limit of 0.04 mg/L test substance was the same as calculated for both calibration curves (Table 3).

The low spike concentration was 0.123 mg/L test substance and high level spike concentration was 8.8 mg/L test substance (Table 4). These concentrations were within the range of test substance concentrations to be used in biological and physical/chemical tests. The volumes of spike solutions (50 ml and 500 ml) used were comparable to the volumes that may be analyzed from physical/chemical and biological studies. The test substance recoveries for the low spike solution ranged between 70.73% and 112.20% with a mean of  $85.91 \pm 22.859\%$ , and for the high spike solution between 97.50% and 111.36% with a mean of  $103.48 \pm 7.121\%$ . (Table 4). The combined mean recovery for low and high spike solutions was  $94.70 \pm 17.943\%$  (Table 4).

The test substance concentration in the method blank was below the method detection limit of 0.04 mg/L isooctyl acrylate.

From the quality assurance standpoint, this test is acceptable because it complies with the acceptance criteria (Table 5).

#### 4.0 CONCLUSIONS

The GC/MS response and test substance, isooctyl acrylate, concentrations between 8.8 and 1,760 mg/L were in linear

correlation. The test substance combined mean recovery (94.70%) from low and high spike solutions suggested that extraction and analytical procedures should be adequate for use with other aqueous samples.

#### 5.0 DEVIATIONS FROM APPROVED ASCI STUDY PLAN

The deviations which occurred while conducting this study were:

- (1) HP model 5890 gas chromatograph and HP model 5970 mass spectrometer were used instead of HP model 5970 gas chromatograph and HP model 5890 mass spectrometer.
- (2) In GC/MS analysis, total inlet purge flow of helium gas was at 40 ml/minute and a septum purge flow was at 1 ml/minute, instead of helium at 5.5 ml/min and a septum purge flow of 5.8 ml/minute.
- (3) In GC/MS analysis, temperature program used was 70°C for 2 minutes and then 8°C/minute to 200°C, instead of 70°C for 2 minutes, and then 8°C/minute to 220°C and holding at 220°C for 2 minutes, or as appropriate. This was because after 180°C nothing eluted from the GC column.

To the best of our current scientific knowledge and understanding,  
this deviation should have no effect on the results presented in  
this report.

6.0 REPORT SIGNATURE

Study Director: rmx Date: 3/26/93  
Minren Xu  
ASCI Corporation/ASCI-Duluth  
Environmental Testing Division

#### 7.0 REFERENCES

Hewlett Packard (HP). 1990. HP 59940A MS ChemStation (HP-UX series) Handbook.

Minitab, Inc. 1988. Minitab Release 6.1. Minitab, Inc., State College, PA.

Organization for Economic Cooperation and Development (OECD). 1981. OECD Guidelines for Testing of Chemicals. OECD Publication Information Center, Washington, DC.

#### 8.0 PERSONNEL INVOLVED IN STUDY AND THEIR RESPONSIBILITIES

Personnel	Responsibility
Minren Xu	Study Director
Connie Coleson	Glassware preparation
Billie Samson	Laboratory assistance
Dinesh Vaishnav	Report preparation
Alan Mozol	QAU
Nancy Jordan	Archivist

Table 1. Isooctyl acrylate (test substance): Solutions for two calibration curves

Test substance solution	Dilution	Test substance nominal concn (mg/L)
Reagent blank	0.0 $\mu$ l test substance in 10 ml acetone (final volume)	0.0
First stock solution	20 $\mu$ l test substance in 10 ml acetone (final volume)	1,760
Second stock solution (SS)	25 $\mu$ l test substance in 25 ml acetone (final volume)	880
Standard solution 1	100 $\mu$ l SS in 10 ml acetone (final volume)	8.8
Standard solution 2	500 $\mu$ l SS in 10 ml acetone (final volume)	44
Standard solution 3	1,000 $\mu$ l SS in 10 ml acetone (final volume)	88
Standard solution 4	5 ml SS in 10 ml acetone (final volume)	440

Table 2. Isooctyl acrylate (test substance): GC/MS responses in two calibration curves

Test substance nominal concn (mg/L)	GC/MS response in first calibration curve	GC/MS response in second calibration curve
Reagent blank	19,622	19,622
1,760	2,719,832,005	2,729,584,720
880	1,390,089,059	1,258,512,351
8.8	22,481,557	10,280,168
44	62,891,391	52,827,478
88	128,917,851	113,808,095
440	658,002,779	622,643,636

Table 3. Isooctyl acrylate (test substance): Statistical analysis of two calibration curves<sup>a</sup>

Parameter	First calibration curve	Second calibration curve
Regression equation	$-1.76e+06 + 1.55e+06 (x)^b$	$-2.48e+07 + 1.54e+06 (x)^b$
Slope $\pm$ SD	$1551104 \pm 12498^c$	$1546151 \pm 29918^c$
Relative standard deviation (% RSD) <sup>d</sup>	0.81%	1.93%
Correlation coefficient (r)	1.000	0.999
Method detection limit (MDL) <sup>e</sup>	0.04 mg/L	0.04 mg/L

<sup>a</sup>GC/MS response and isooctyl acrylate (test substance) concentration (milligrams per liter) were plotted on ordinate and abscissa, respectively.

<sup>b</sup>Equation was generated using MS ChemStation software (HP 1990).

<sup>c</sup>Slope and SD were calculated using Minitab<sup>®</sup> statistical software (Minitab, Inc. 1988), as HP-UX software did not calculate SD.

<sup>d</sup>Percentage RSD = (Standard deviation of slope/slope) X 100.

<sup>e</sup>MDL = 3 X response in reagent blank (= 19,622; Table 2)/slope.

Table 4. Isooctyl acrylate (test substance): Recoveries from spiked deionized water.

Type of solution	R # p	Test substance target concn (mg/L)	Test substance measured concn (mg/L)*	% Re- covery (R) <sup>b</sup>	Mean $\pm$ SD% recovery (R) <sup>c</sup>
Method blank	1	0.0	<0.04 <sup>d</sup>	-	-
Low spike	1	0.123	0.092	74.80	85.91 $\pm$ 22.859
	2	0.123	0.138	112.20	
	3	0.123	0.087	70.73	
High spike	1	8.8	8.58	97.50	103.48 $\pm$ 7.121
	2	8.8	8.94	101.59	
	3	8.8	9.80	111.36	
Combined recovery from low spikes + high spikes					94.70 $\pm$ 17.943

<sup>a</sup>Determined using first calibration curve (Table 3).

<sup>b</sup> $R_p = (\text{Measured concentration}/\text{Target concentration}) \times 100$ .

<sup>c</sup>Mean R was calculated using  $R_p$  values which fell within  $R \pm 3SD$  range.

<sup>d</sup>Method detection limit (MDL) was 0.04 mg/L isooctyl acrylate.

Table 5. Isooctyl acrylate (test substance): QA criteria and test acceptability

QA criterion	Results
Relative standard deviation of calibration curve (% RSD) must be within 10%	% RSD of first calibration curve was 0.81% and of second calibration curve was 1.93%
Post run standard response must be within 10% of the same standard analyzed at the beginning of the test	Responses from all peaks from post run standard differed by 5.95% compared to the beginning of the test

**CERTIFIED COPY**

Signature: [Signature] Date: 3/26/93